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BIOLOGICAL TREATMENT OF GROUNDWATER CONTAMINATED WITH MIXTURES OF AROMATIC COMPOUNDS

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This report presents the results of a 3-week preliminary field demonstration using an aboveground fixed-film bioreactor to biodegrade chlorinated aromatic compounds. The site at Kelly AFB, TX is an abandoned waste storage area where the soil and groundwater is contaminated with a mixture of chlorinated solvents. Contaminant removal by a reactor colonized with Pseudomonas sp. JS150 was compared to that of a reactor colonized with indigenous groundwater bacteria. Other process measurements included temperature, pH, dissolved oxgen, and fluctuations in microbial populations. Both biofilm populations degraded the organic compounds when the reactors were operated at a hydraulic residence time of 40 minutes. The concentrations of volatile organic compounds were reduced from the mg/l to the low ug/l range when sufficient dissolved oxygen was maintained. These results indicate that high removal efficiencies for complex mixtures of organic compounds can be achieved by immobilized bacterial populations.

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EXECUTIVE SUMMARY

A. OBJECTIVE

The objective of this pilot-scale field study was to determine the ability of an aboveground fixed-film bioreactor to degrade mixtures of aromatic compounds in groundwater. Another objective of this study was to test whether inoculation of the bioreactor with a pure culture of degradative bacteria improved the performance over inoculation with an indigenous microorganism.

B. BACKGROUND

The Air Force is faced with the cleanup of over 2,600 contaminated sites identified under the Installation Restoration Program (IRP). Approximately 800 of these sites involve groundwater contaminated with various types of solvents. Current technologies for treating contaminated groundwater include air stripping and carbon sorption. However, advances in the development of fixed-film bioreactors have furthered the use of bioremediation in groundwater treatment. Bioremediation completely destroys toxic contaminants, whereas air stripping and carbon sorption merely transfer the contaminants between phases.

C. SCOPE

A 3-week field demonstration was conducted at Site S1 at Kelly AFB, Texas. Laboratory- and pilot-scale submerged fixed-film bioreactors were tested with groundwater containing benzene, naphthalene, phenol, toluene, ortho and para-cresol, 2,4-dimethylphenol, chlorobenzene and ortho, meta, and para-dichlorobenzene. Preliminary tests were conducted under a variety of operating conditions and temperature, pH, dissolved oxygen, and fluctuations in microbial populations were monitored.

D. RESULTS

Laboratory experiments indicated that Pseudomonas strain JS150 could aerobically biodegrade complex mixtures of solvents if appropriate dissolved oxygen levels were maintained. After the initial 5 days of continuous-flow operation, JS150 was the predominant organism in the bioreactor biofilm but was displaced by indigenous bacteria from the groundwater after 10 days.

In the field, one pilot-scale bioreactor was inoculated with microorganisms indigenous to groundwater at Site S1, Kelly AFB and the other bioreactor was inoculated with a pure culture of Strain JS150. In a continuous-flow experiment, both biofilm populations reduced concentrations of benzene, toluene, chlorobenzene, and dichlorobenzene from the parts per million level down to the parts per billion level when the reactor was operated at steady state with a residence time of 40 minutes. The bioreactor inoculated with JS150 showed greater removal efficiencies for the more highly substituted aromatic compounds.

Several chlorinated solvents previously considered to be recalcitrant were readily degraded.

E. CONCLUSIONS

These results indicate that an aerobic fixed-film process was very effective in degrading mixtures of toxic aromatic compounds. The pure culture JS150 was more efficient at removing highly substituted compounds such as para-dichlorobenzene, however JS150 was displaced by indigenous microorganisms at Site S1 following continuous-flow operation. The indigenous microorganisms were capable of degrading mixtures of chloro- and methylaromatic compounds which suggests that biodegradation may be slowly removing the contaminant *in situ*, without intervention. Results indicate that inoculation of a pure culture would not be necessary in this situation but it might be required in situations involving recent contamination of chlorinated aromatic compounds.

F. RECOMMENDATIONS

Based on the laboratory and field study results, it was concluded that all the contaminants at Site S1 can be biodegraded by indigenous microorganisms. Therefore, it is recommended that bioremediation be considered for cleanup of Site S1. A second field effort is being initiated at an Air Force contamination site to collect additional operating data to be used to design a full-scale system.

PREFACE

This report was prepared by the Air Force Civil Engineering Support Agency, Civil Engineering Laboratory, Tyndall AFB Fl 32403-6001. This report summarizes work done between 1 August 1990 and 15 December 1990. The HQ AFCESA/RAVW project officer was Capt Catherine M. Vogel.

This report has been reviewed by the Public Affairs Office and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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SECTION I
INTRODUCTION

A. OBJECTIVE

The goal of this pilot-scale investigation was to develop a fixed-film aboveground bioreactor capable of treating groundwater or waste streams contaminated with mixtures of chlorinated aromatic compounds. Due to the need for a cost-effective, destructive alternative treatment method, researchers at the Civil Engineering Support Agency developed a biological process which readily destroys mixtures of aromatic compounds. This project was a preliminary field test to determine whether this biological system could degrade mixtures of compounds previously thought to be recalcitrant in the field.

B. BACKGROUND

The Air Force is faced with the clean up of over 2,600 contamination sites identified under the Installation Restoration Program (IRP). Approximately 800 of these sites involve groundwater contaminated with various types of solvents. The biological removal of organic compounds in wastewater has been used for many years in conventional aerobic treatment processes. The application of these treatment methods to groundwater contaminated with organic wastes has often been limited by low concentrations of organic compounds, rapid loss of volatile contaminants via air stripping, and the lack of retention of an active biomass (1). Despite the potential for complete destruction of organic compounds by biological treatment, conventional groundwater remediation processes have often used nondestructive technologies such as air stripping and carbon adsorption (2). These technologies merely transfer the contaminant from one phase to another. However, advances in the understanding of biofilm processes (3,4), and the development of fixed-film bioreactors (5,6,7) have facilitated the application of biodegradation processes to groundwater treatment.

Hazardous wastes containing xenobiotic compounds are commonly refractory to biodegradation due to their toxicity and absence of microorganisms able to degrade the compounds. One approach is to use microorganisms selected for their ability to degrade specific chemicals. The efficacy of applying selected bacterial strains for the remediation of hazardous compounds has been demonstrated in soils contaminated with pentachlorophenol (8), 2,4,5-trichlorophenoxyacetic acid (9), and 2,4-dichlorophenoxyacetic acid (10). The performance of wastewater treatment processes have also been enhanced by the addition of bacterial strains selected for the ability to degrade pentachlorophenol (11), chlorophenols (12), and s-triazenes (13). While these studies have reported beneficial effects associated with the inoculation

of specific bacterial strains (14), no enhancement associated with addition of a chlorobenzoate degrader to an activated sludge unit has been found. However, in a more recent report (15), enhanced performance was observed when the inoculated bacterial strain was selected for its metabolic potential and its adaptation to environmental conditions.

The pseudomonads have long been noted for their metabolic versatility (16,17). The list of compounds that can be degraded by pseudomonads includes an increasing number of hazardous compounds. One of the most versatile strains, Pseudomonas sp. Strain JS1, was originally isolated from sewage samples for its ability to degrade para-dichlorobenzene (18). Derivatives of this strain, Strains JS6 and JS150, have been shown to degrade numerous other aromatic compounds (19,20,21). These strains can also degrade mixtures of chloro- and methyl-substituted aromatic substrates (22), which are generally considered not to be degraded simultaneously (23). Because Strain JS150 can synthesize at least four ring-fission pathways and three separate dioxygenases, we have investigated the biodegradation of complex mixtures of aromatic compounds by this strain.

C. SCOPE

After extensive fundamental research on the metabolic capabilities of Strain JS150, the concept was tested in the field to determine its applicability to Air Force pollution problems. This study involved the use of submerged fixed-film bioreactors in laboratory and field investigations of the biological treatment of groundwater contaminated with hazardous wastes. We conducted a field investigation at Kelly Air Force Base, Texas, where past storage of waste solvents resulted in contamination of the alluvial sediment soil and the unconfined shallow aquifer (24). The groundwater was contaminated with a mixture of chloro- and methylaromatic compounds including toluene, ortho and para-cresol, 2,4-dimethylphenol, chlorobenzene, ortho, meta, and para-dichlorobenzene, as well as acetone, benzoate, naphthalene, and trichloroethylene.

Bioreactors colonized with either Strain JS150 or indigenous groundwater bacteria were tested for their ability to biodegrade the contaminants under continuous-flow conditions. Preliminary tests were conducted on groundwater at the site for 3 weeks under a variety of operating conditions.

One objective of this investigation was to determine whether a fixed-film bioreactor system performed better with a biofilm established with a specific bacterial strain or with mixtures of indigenous groundwater bacteria. Based on the "decay theory" of biological treatment for dilute organic wastes (5), we hypothesized that an established biofilm containing Strain JS150 might persist despite the potential competition with indigenous groundwater bacteria.

Results from this field effort are being evaluated for use in the IRP feasibility study for the clean up of this site. Plans are being initiated for a second field test at an Air Force contamination site to collect additional operating data for use in the design of a full-scale system. Final design and operating specifications and guidance are expected to be available in 1994.

SECTION II

MATERIALS AND METHODS

A. LABORATORY PROCEDURES

Pseudomonas sp. JS150 has been previously described (21). Indigenous groundwater bacteria were obtained from Site S1 at Kelly AFB (24), by pumping unfiltered groundwater to bioreactors operated in a continuous recycle mode. Chlorobenzene degrading biofilms were established on bioreactor carrier material by operating the bioreactors in a batch "fill-and-draw" mode with chlorobenzene provided in the gas phase. Batch cultures were grown at 23°C in a mineral salts medium. The medium (pH 7.2) was diluted 1:1 with tap water and replaced at 24-hour intervals (17). The initial chlorobenzene concentration was 0.1 mg/L in the air and was gradually increased to 2.0 mg/L at an air flow of 0.2 L/min per kg of carrier material.

B. SYSTEMS DESIGN AND OPERATION

1. Bench-Scale Bioreactor Design and Operation

A laboratory bioreactor was constructed with a glass chromatographic column (60 x 5cm; Ace Glass, Vineland, N.J.), filled with 0.9 kg (1.2 l) Celite® Biocatalyst Carrier R-635 (Manville, Denver, CO). The laboratory bioreactor was inoculated with Strain JS150 as described above. The configurations of the laboratory bioreactor and the continuous-flow system were the same as described previously by Bouwer et al (4). These experiments were conducted with mineral salts broth (MSB) (pH 7.0) diluted 1:9 with deionized water pumped at 16.7 mL/min (30 minutes hydraulic residence time). Water-saturated chlorobenzene was added via a syringe pump such that the input concentration was between 1 and 2 mg/L. Additional experiments with contaminated groundwater were performed by mixing the dilute MSB (pH 7.0) with groundwater (at a ratio of 3:1). These experiments were run at flow rates corresponding to residence times of 30, 40, and 50 minutes. Groundwater was pretreated by pumping through a water softener (model 9000; Continental, Oklahoma City, OK) and a 100 μm filter (Filterite®, Timonium, MD.). When the total input concentration of aromatic compounds exceeded 5 mg/L the MSB reservoir was sparged with oxygen to provide sufficient dissolved oxygen to the bioreactor. The input and effluent concentrations of purgeable aromatic compounds were determined by gas chromatography (GC) analysis using EPA Method 602. Water soluble aromatic compounds were determined by high-performance liquid chromatography (HPLC).

2. Pilot-Scale Bioreactor Design and Operation

Two pilot-scale bioreactors were used in field studies at Kelly AFB (Figure 1). Stainless steel columns (183 x 30.4 cm) were packed with approximately 90 kg of Celite® R-635 Biocatalyst Carrier. The pilot-scale bioreactors were originally built by Battelle, Columbus, Ohio and used as a dual-stage trickling filter bioreactor for the degradation of trichloroethylene (TCE) in a field demonstration at Tinker AFB, Okla. (25). These bioreactors were reconfigured as upflow submerged fixed-film bioreactors. Because the groundwater at Site S1 contained a significant concentration of iron, calcium, and magnesium (24), it was treated by passage through an in-line water softener (model 9000; Continental, Oklahoma City, Okla.). The groundwater was then diluted with tap water and pH, inorganic nutrients, dissolved oxygen, and temperature were adjusted as required. The pH and inorganic nutrient concentrations were adjusted by addition of a nutrient solution (K_2HPO_4, 450 g/L; KH_2PO_4, 50 g/L; NH_4Cl, 37.5 g/L; pH 8.5) at a rate of 15 μL/L groundwater. Dissolved oxygen levels were initially maintained by addition of oxygen at low flow rates to a stainless steel holding tank (50 liters). The oxygen delivery system was changed during later phases of the project and air was pumped directly to the bioreactor at a rate of 150 mL/min. In addition, a continuous recycle pump was installed when the oxygen delivery system was changed. The pump recycled part of the flow from the lower half of the bioreactor where the majority of the contaminant and oxygen removal occurred.

One bioreactor was inoculated with microorganisms indigenous to groundwater at Site 1, Kelly AFB. The reactor was operated in the continuous recycle mode for 4 days, then operated in the "fill-and-draw" mode for 7 days. The second bioreactor was inoculated with 15 liters of a pure culture of Strain JS150 in the laboratory before being transported to Kelly AFB. The reactor was operated in the "fill-and-draw" mode for 4 days, drained, and the JS150 biofilm was kept moist and aerated during transport to Kelly AFB. Upon arrival at Kelly AFB the reactor was operated in the "fill-and-draw" mode for an additional 4 days before continuous operation was started. Both pilot-scale bioreactors and the field laboratory were housed and transported in a 40-foot closed trailer. The laboratory was equipped for performing both microbiological and analytical chemistry measurements. During continuous-flow operation the input and effluent concentrations of contaminants were determined as described below.

C. SAMPLING AND ANALYSIS

Samples to be analyzed for volatile organic compounds were collected directly from the input and effluent ports of the bioreactors with a 10 mL gas tight syringe (Hamilton, Reno, NV). No gas headspace was allowed in these samples and 5 mL subsamples

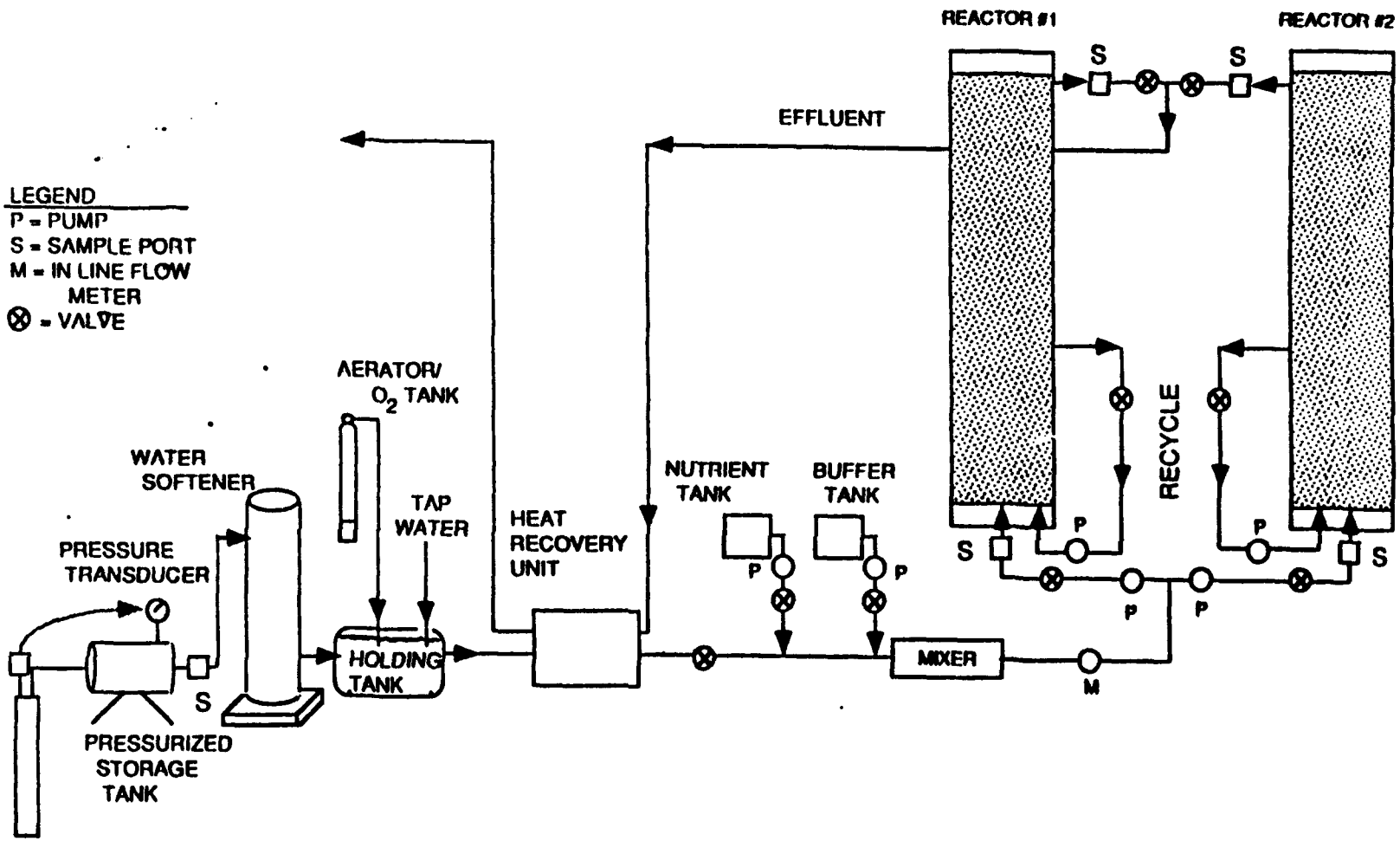

Figure 1. Schematic Diagram of the Pilot-Scale Bioreactor Used at Site S1 Kelly AFB.

were immediately analyzed by GC using EPA Method 602. GC was done on a 10-foot (304.8 cm) stainless steel packed column (10 percent SP-1000, 80/100 Supelcoport®; Supelco. Inc., Bellefonte, Penn.) in a Hewlett-Packard 5890 GC equipped with a photoionization detector (HNU Systems, Inc., Newton, Mass.). The purge and carrier gas was high-purity helium. Samples were purged for 12 minutes at 40 mL/min and trapped on Tenax® (Alltech Assoc., Deerfield, IL). The Tenax® traps were then heated to 160°C for 4 minutes and the carrier gas flow rate was 42 mL/min. Oven temperature was initially held at 70°C for 3 minutes and then increased at a rate of 12°C/min to a final temperature of 140°C, and the detector temperature was maintained at 220°C. HPLC was performed on a u-Bondapak® C_{18} column (3.9 mm by 30 cm; Waters Associates, Inc., Milford, Mass.) with methanol-water-phosphoric acid (370:630:1) as the mobile phase at a flow rate of 1.5 mL/min. Compounds were detected by the UVA_{210}, A_{254}, and A_{280} with a model 1040A diode array detector (Hewlett-Packard Co., Palo Alto, CA). Groundwater samples collected at the start and end of the field experiments were analyzed for volatile organic compounds and inorganic compounds by Professional Service Industries, Inc., San Antonio, Tex.

The dissolved oxygen levels in the bioreactor samples were measured with a platinum electrode and portable monitors (Model 58; Yellow Springs Instrument Co., Yellow Springs, OH). The pH was measured with a glass electrode and portable monitors (Model 601A; Orion Research, Cambridge, Mass).

Pellets were aseptically removed from the input and effluent ends of the laboratory and pilot-scale bioreactors during the experiments. The pellets were crushed and ground with mortar and pestle for 1 minute and diluted in MSB. Appropriate dilutions were spread on nutrient agar (Difco, Detroit, Mich.) and MSB plates incubated under a chlorobenzene atmosphere at room temperature. The proportion of chlorobenzene degraders that were JS150 were estimated by replica plating on MSB plates incubated under a naphthalene atmosphere.

SECTION III

SITE DESCRIPTION

Site S1, at Kelly AFB, is located near the northern installation boundary, northeast of Building 1573 (Figure 2). This area was used for storage of waste, solvents and transformers from the early 1960s to 1973 when it was abandoned and regraded. During the loading and unloading of wastes, spills collected in the depression/sump area described in Figure 2. The soil and shallow groundwater associated with this area were contaminated and have subsequently been designated an Installation Restoration Program priority site by the Air Force. Senko et al. (24) documented the hydrogeology of the site. The contaminated soils of Site S1 were described as clay loam, loam, and gravel alluvial sediments that were poorly drained and possessing low permeabilities. The contaminated groundwater at Site S1 is 7.6-10.4 meters deep with the lower boundary composed of a Navarro clay aquitard. Localized high points in the Navarro clay topography, sometimes restrict lateral flow of the groundwater and dry zones associated with this emergent surface have been noted (24). The groundwater generally flows to the northeast, resulting in a contaminant plume that extends past the Kelly AFB boundary.

Our interest in Site S1 was initially based on previous reports of a groundwater plume contaminated primarily with chlorobenzene and smaller amounts of other relatively water-soluble aromatic compounds such as benzene (24). Our original question was whether Strain JS150 and other bacterial strains could biodegrade low concentrations of these hazardous compounds in fixed-film bioreactors. The preliminary laboratory experiments and design of the bioreactor system were based on the assumption that the total concentration of aromatic compounds would be around 1-5 mg/L. The concentration of contaminants was an order of magnitude higher than expected (Table 1). Because of dry zones associated with the emergent Navarro clay aquitard and because of logistical constraints, the well was located within the previous sump area rather than down gradient in the chlorobenzene plume (Figure 2). The mixture of contaminants in the groundwater was unexpectedly complex (Table 1). These observations dictated that the experimental plan be altered and provided the opportunity to investigate the simultaneous biodegradation of complex mixtures of aromatic compounds at a pilot scale. Bench-scale research on this topic was already in progress (21,22).

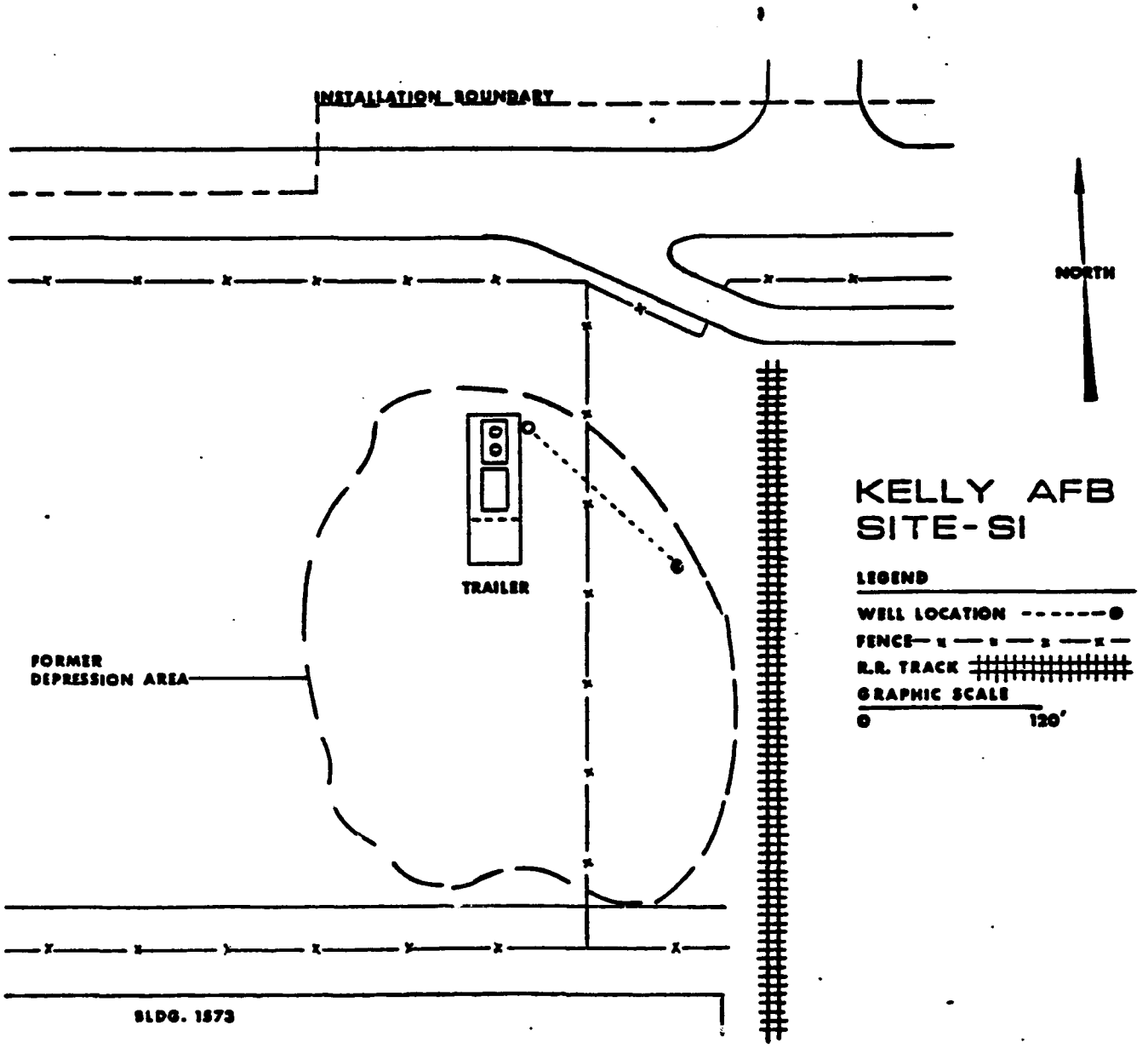

Figure 2. Map of Site S1, Kelly AFB, and Layout of Pilot-Scale Bioreactor .

TABLE 1. CONCENTRATIONS OF ORGANIC COMPOUNDS IN SITE S1 GROUNDWATER.

Compound	Concentration (mg/l)
Benzene	0.96
Benzoic Acid	0.79*
Chlorobenzene	6.52
o-Dichlorobenzene	12.39
m-Dichlorobenzene	0.36*
p-Dichlorobenzene	1.24
2,4-Dimethylphenol	4.00*
2-Methylphenol	2.40*
4-Methylphenol	2.90*
Naphthalene	0.10*
Phenol	0.20*
Toluene	0.63

*Results provided by PSI, Inc.

SECTION IV

RESULTS

A. LABORATORY EXPERIMENTS

A continuous-flow experiment was conducted for 15 days following an initial 10-day establishment of a JS150 biofilm in the laboratory bioreactor (see Appendix A for summary of raw data). The hydraulic residence time was held constant at 30 minutes for 10 days. Chlorobenzene was the sole contaminant for the first 5 days then contaminated groundwater (25 percent) was continuously supplied during the second 5 days.

The biodegradation of chlorobenzene by the JS150 biofilm resulted in effluent concentrations of chlorobenzene well below the 100 µg/L EPA drinking water limit during the first 5 days of the experiment (Figure 3). The operating temperature during the first 5 days varied between 12 and 22°C with no detectable changes in the efficiency of chlorobenzene removal by the JS150 biofilm. The temperature was maintained at 20°$\pm$ 2°C during the remainder of the experiment. During the second 5 days the effluent chlorobenzene concentrations rose to around 400 µg/L which represented approximately a 90 percent removal of the input chlorobenzene (Figure 3). This experiment demonstrated that the maintenance of appropriate dissolved oxygen was required for the biodegradation of the mixed aromatic compounds. In control experiments where the oxygen supply to the dilution feedstock was switched with argon the effluent chlorobenzene concentration rose to 89 percent of the input concentration after 5 hours (Figure 3). When the oxygen was added again the effluent chlorobenzene concentration fell to previous levels. These results indicate that aerobic biodegradation is the major removal mechanism in these experiments and that abiotic losses play a relatively minor role.

Following the 10-day operation with a 30 minute hydraulic residence time, the laboratory bioreactor was run an additional 5 days with the groundwater mix pumped at longer residence times (40 and 50 minutes). These changes resulted in enhanced removal of all the mixed aromatic contaminants except <u>para</u>-dichlorobenzene in the site S1 groundwater (Table 2). The continuous pumping of this groundwater feed resulted in the deposition of an orange scale on the pellets which was believed to be largely composed of oxidized iron and mineral salts deposits. The formation of this scale may have reduced the surface area of the pellets and the associated biofilm, and may have been responsible for the decreased chlorobenzene removal efficiency described in Figure 3. However, the presence of the complex mixture of aromatic compounds, especially high concentrations of substrates such as <u>ortho</u>-dichlorobenzene and 2,4-dimethylphenol, may have also played a role in the decreased removal efficiency.

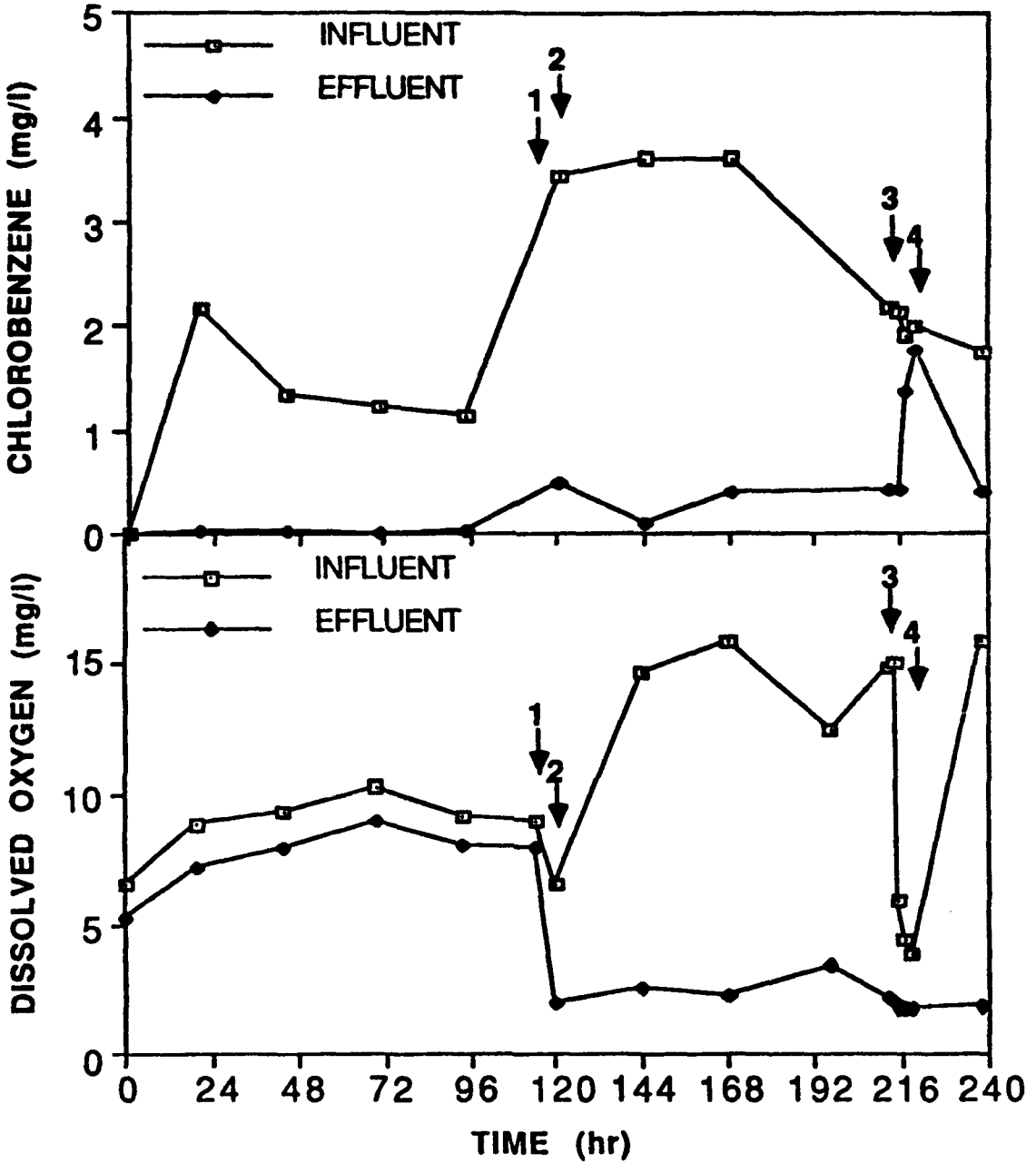

Figure 3. Chlorobenzene Removal in the Bench-Scale Bioreactor Run Continuously at a 30-Minute Hydraulic Residence Time. The influent initially contained chlorobenzene as sole carbon source but was changed to 25% groundwater after 5 days (Arrow 1). Dissolved oxygen in the feedstock was increased by sparging oxygen (Arrows 2 and 4) and decreased by sparging argon (Arrow 3) into the dilution medium.

TABLE 2 BIODEGRADATION OF ORGANIC COMPOUNDS IN SITE S1 GROUNDWATER IN THE JS150 LABORATORY BIOREACTOR OPERATED AT DIFFERENT HYDRAULIC RESIDENCE TIMES.

Compound	% Removal		
	Hydraulic Residence Time		
	30 min.	40 min.	50 min.
Benzene	69	93	87
Chlorobenzene	89	91	92
o-Dichlorobenzene	40	66	74
p-Dichlorobenzene	91	22	67
2,4-Dimethylphenol	7	19	58
o & *p*-Cresol	34	36	56
Toluene	83	85	84
Trichloroethylene	5	89	93

An analysis of the microbial composition of the laboratory bioreactor biofilm was performed by dilution spread plate methods. The results presented in Table 3 indicate that Strain JS150 was the predominant member of this biofilm after the initial 5 days of continuous-flow operation. However, after 10 days operation with the groundwater feed, Strain JS150 only represented approximately 14 percent of the chlorobenzene degraders in the biofilm. Based on these results, Strain JS150 seems to have been displaced by microorganisms indigenous to site S1 groundwater. This result may explain why para-dichlorobenzene removal was not enhanced when the hydraulic residence time in the laboratory bioreactor was increased. The "decay-mode" hypothesis does not appear to apply to the relatively high substrate concentrations used in these experiments. Perhaps at lower substrate concentrations, where bacterial growth is not supported, the JS150 biofilm population would have persisted.

B. PILOT-SCALE EXPERIMENTS

After biofilm populations were established in the two pilot-scale bioreactors, a continuous-flow experiment was run for 10 days at Kelly AFB (see Appendix B for summary of raw data). The operating conditions for this experiment are shown in Table 4. The hydraulic residence time was held constant at 40 minutes (112 L/h) for 7 days but the first 5 days of continuous operation were not at steady state because the groundwater flow was sporadic. The low permeability of the soil and the concomitant slow recharge of the groundwater at Site S1 only allowed for continuous pumping of approximately 0.5 L/min from the well. Steady state was achieved after 5 days by pumping groundwater diluted 1:7 with tap water at a hydraulic residence time of 40 minutes. Although the groundwater supply was problematical, the addition of tap water to maintain continuous operation did dilute the high concentration of contaminants in site S1 groundwater. The results shown in Table 5 were obtained at the end of 7 days continuous operation at the 40-minute hydraulic residence time. Both the bioreactor colonized with JS150 and the bioreactor colonized with indigenous microorganisms from Kelly AFB (KAFB) removed most of the aromatic compounds from the contaminated groundwater. However, the bioreactor originally colonized with JS150 showed greater removal efficiencies for the more highly substituted aromatic compounds (Table 5).

Following the initial 7-day operation with a 40-minute hydraulic residence time, the pilot-scale bioreactor originally colonized with JS150 was operated an additional 3 days at longer residence times (1 day at 20 minutes and 2 days at 30 minutes). The increased groundwater flow was achieved by turning off the second bioreactor. The input and effluent concentrations of benzene, trichloroethylene, toluene, chlorobenzene, ortho and para-dichlorobenzene are presented in Figures 4-9. The steady state results for both bioreactors indicate efficient removal of these aromatic compounds when the residence time was maintained

TABLE 3. ENUMERATION OF BACTERIA FROM THE BENCH-SCALE BIOREACTOR. Total counts (nutrient agar) and chlorobenzene degraders were measured in samples from the bench-scale bioreactor.

Sample	CFU (x 10^6) per gram		
	Nutrient agar	Chlorobenzene	Naphthalene(%)
To			
bottom	1340	1250	93
top	1320	1190	91
T5d			
bottom	1170	437	80
top	660	365	77
T15d			
bottom	1630	26	16
top	280	6	11

TABLE 4. PILOT-SCALE BIOREACTOR OPERATING CONDITIONS AT SITE S1.

Parameter	Date 11/27/90-12/2/90[a]	12/3/90	12/4/90	12/5/90	12/6/90	12/7/90	12/8/90
Reactor #1(JS150)							
Hydraulic residence time (min.)	40.00	40.00	40.00	40.00	20.00	30.00	30.00
Groundwater dilution	0.10 - 0.50	0.14	0.14	0.14	0.14	0.14	0.14
Air flow rate (ml/min.)	0.00[b]	150.00	150.00	150.00	150.00	150.00	150.00
Nutrient addition (mg PO_4/l)	8.00	8.00	8.00	0.00	0.00	0.00	0.00
Reactor #2(KAFB)							
Hydraulic residence time (min.)	40.00	40.00	40.00	40.00	-	-	-
Groundwater dilution	0.10- 0.50	0.14	0.14	0.14	-	-	-
Air flow rate (ml/min.)	0.00[b]	150.00	150.00	150.00	-	-	-
Nutrient addition (mg PO_4/l)	8.00	8.00	8.00	8.00	-	-	-

[a] Non-steady state operation.
[b] O_2 added to holding tank.

TABLE 5. BIODEGRADATION OF AROMATIC COMPOUNDS BY BACTERIAL POPULATIONS COLONIZING THE PILOT-SCALE BIOREACTOR.

Compound	Influent concentration (mg/l)	% Removal	
		JS150	KAFB
Benzene	0.145	99	98
Chlorobenzene	0.842	95	87
o-Dichlorobenzene	0.834	59	23
p-Dichlorobenzene	0.108	79	10
2,4-Dimethylphenol	0.200[b]	93	69
2-Methylphenol	0.170[b]	100	89
4-Methylphenol	0.170[b]	100	100
Toluene	0.172	92	82

[a] Results obtained after 7 days operation at 40 minutes residence time.

[b] Results provided by PSI Inc.

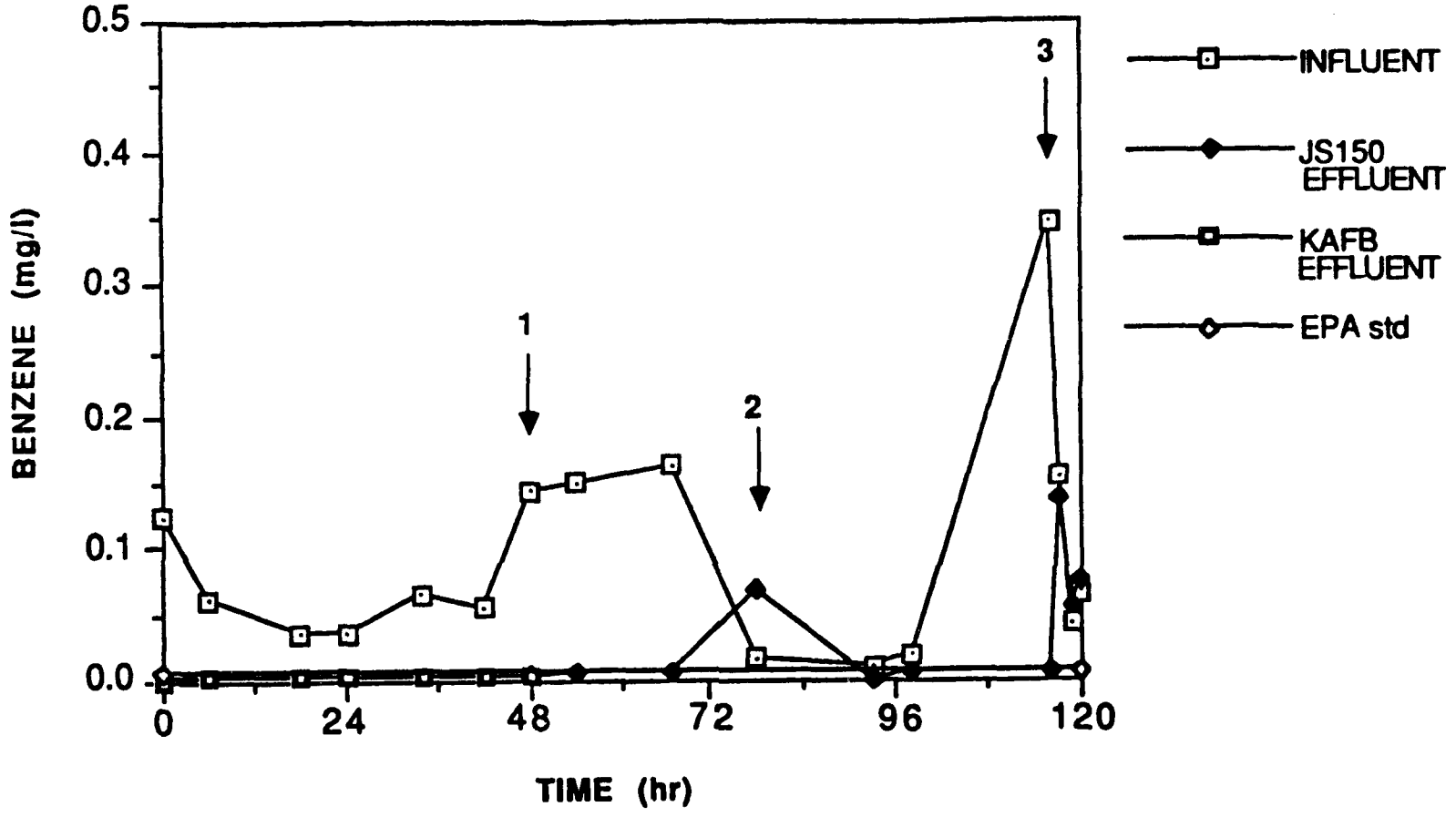

Figure 4. Benzene Removal in the Pilot-Scale Bioreactors. Bioreactors were colonized with either Strain JS150 or the bacteria indigenous to Kelly AFB (KAFB) and operated at 40 minute, 20 minute (Arrow 1), and 30 minute hydraulic residence times (Arrow 2). Dissolved oxygen levels in the feedstock were reduced by turning off air flow to the bioreactor and sparging the holding tank with helium (Arrow 3).

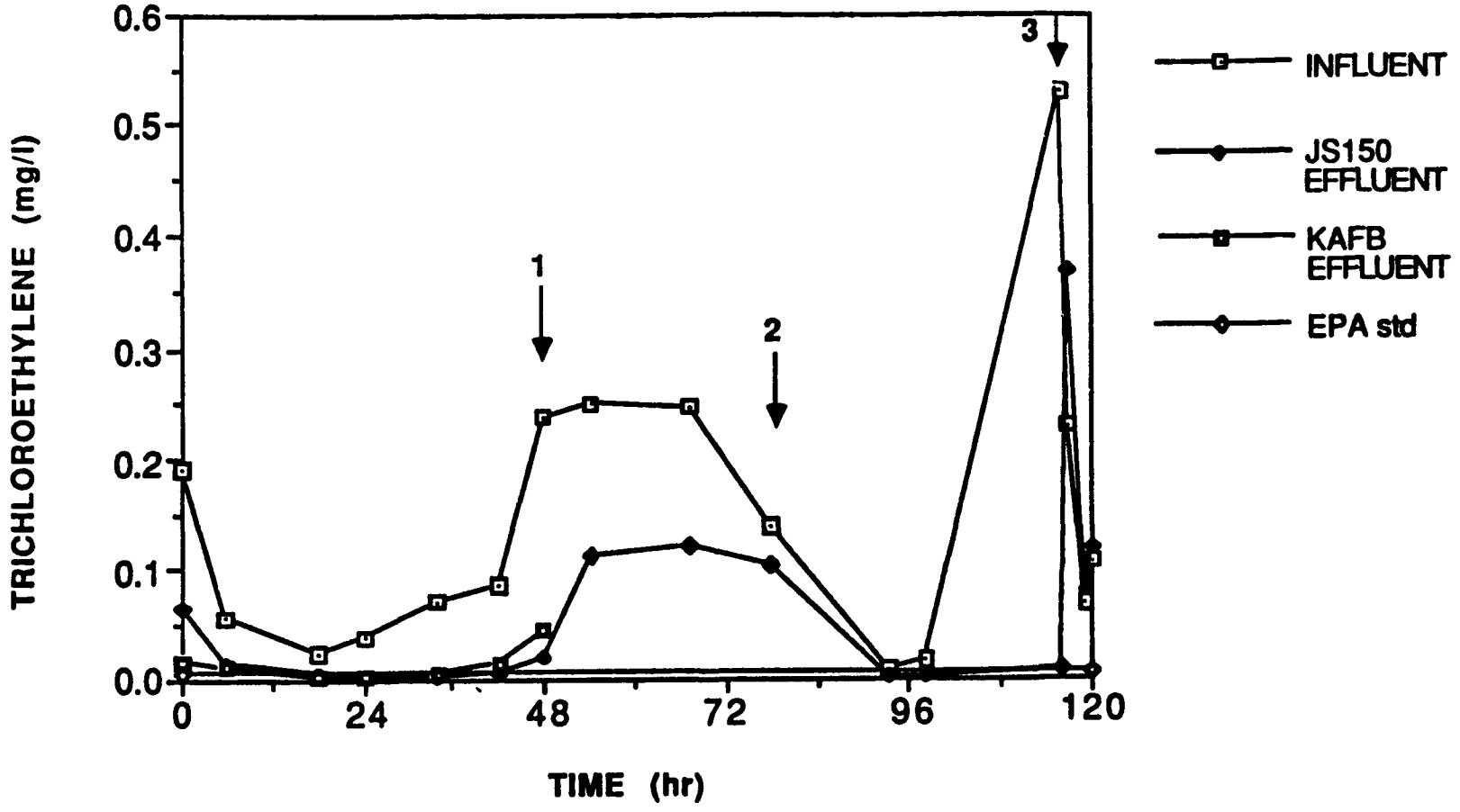

Figure 5. Trichloroethylene Removal in the Pilot-Scale Bioreactors. Bioreactors were colonized with either Strain JS150 or the bacteria indigenous to Kelly AFB (KAFB) and operated at 40 minute, 20 minute (Arrow 1), and 30 minute hydraulic residence times (Arrow 2). Dissolved oxygen levels in the feedstock were reduced by turning off air flow to the bioreactor and sparging the holding tank with helium (Arrow 3).

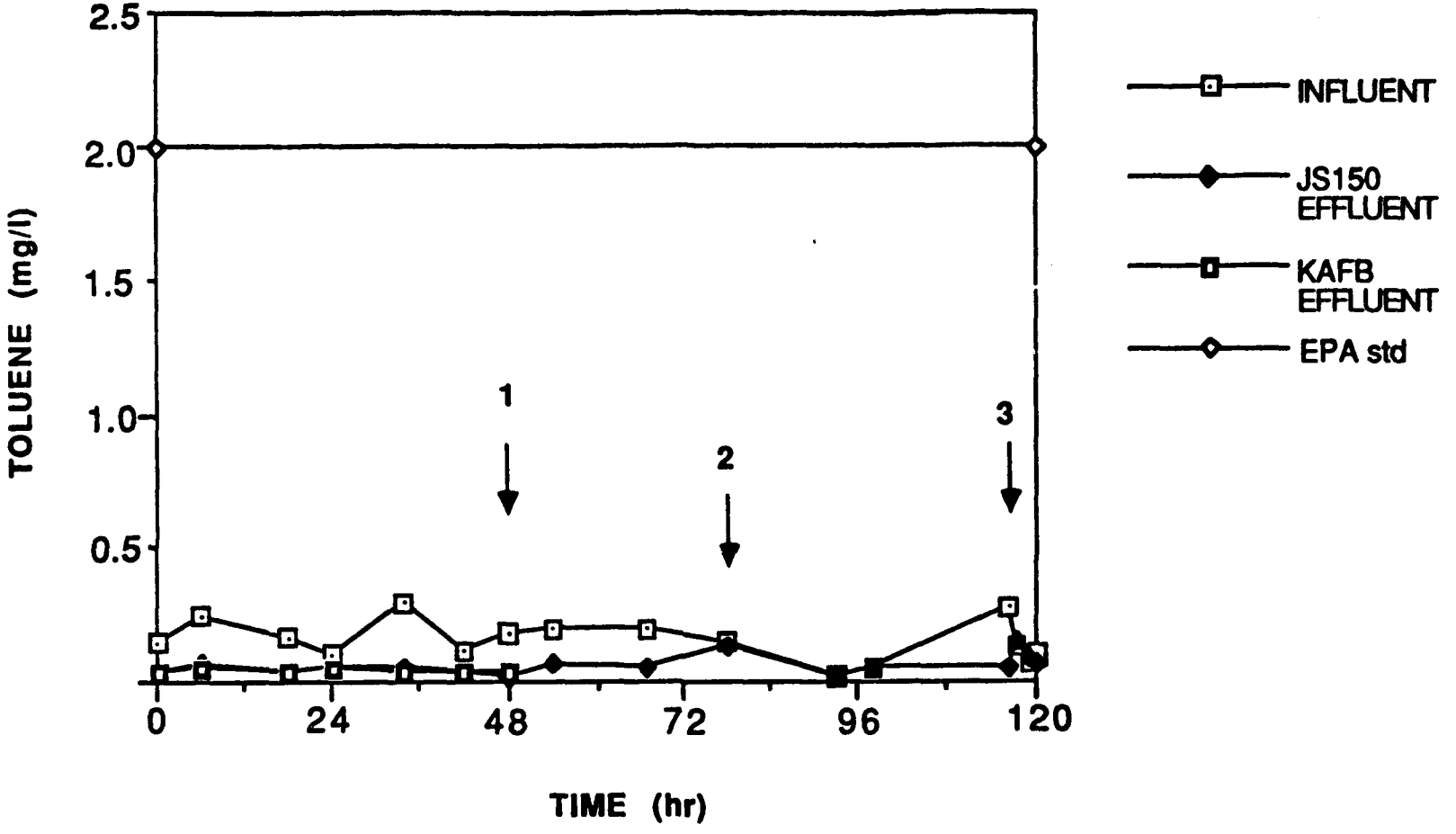

Figure 6. Toluene Removal in the Pilot-Scale Bioreactors. Bioreactors were colonized with either Strain JS150 or the bacteria indigenous to Kelly AFB (KAFB) and operated at 40 minute, 20 minute (Arrow 1), and 30 minute hydraulic residence times (Arrow 2). Dissolved oxygen levels in the feedstock were reduced by turning off air flow to the bioreactor and sparging the holding tank with helium (Arrow 3).

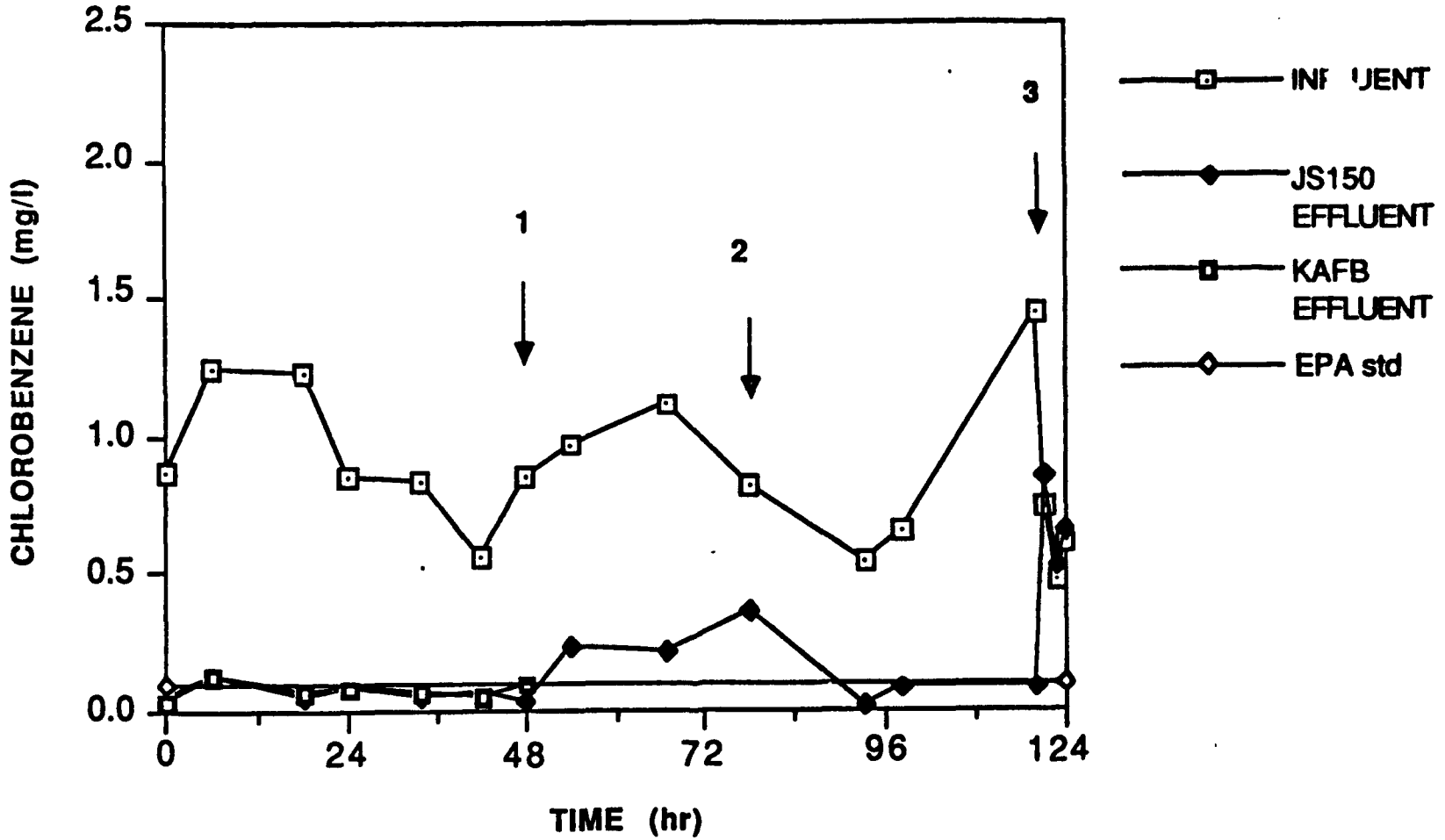

Figure 7. Chlorobenzene Removal in the Pilot-Scale Bioreactors. Bioreactors were colonized with either Strain JS150 or the bacteria indigenous to Kelly AFB (KAFB) and operated at 40 minute, 20 minute (Arrow 1), and 30 minute hydraulic residence times (Arrow 2). Dissolved oxygen levels in the feedstock were reduced by turning off air flow to the bioreactor and sparging the holding tank with helium (Arrow 3).

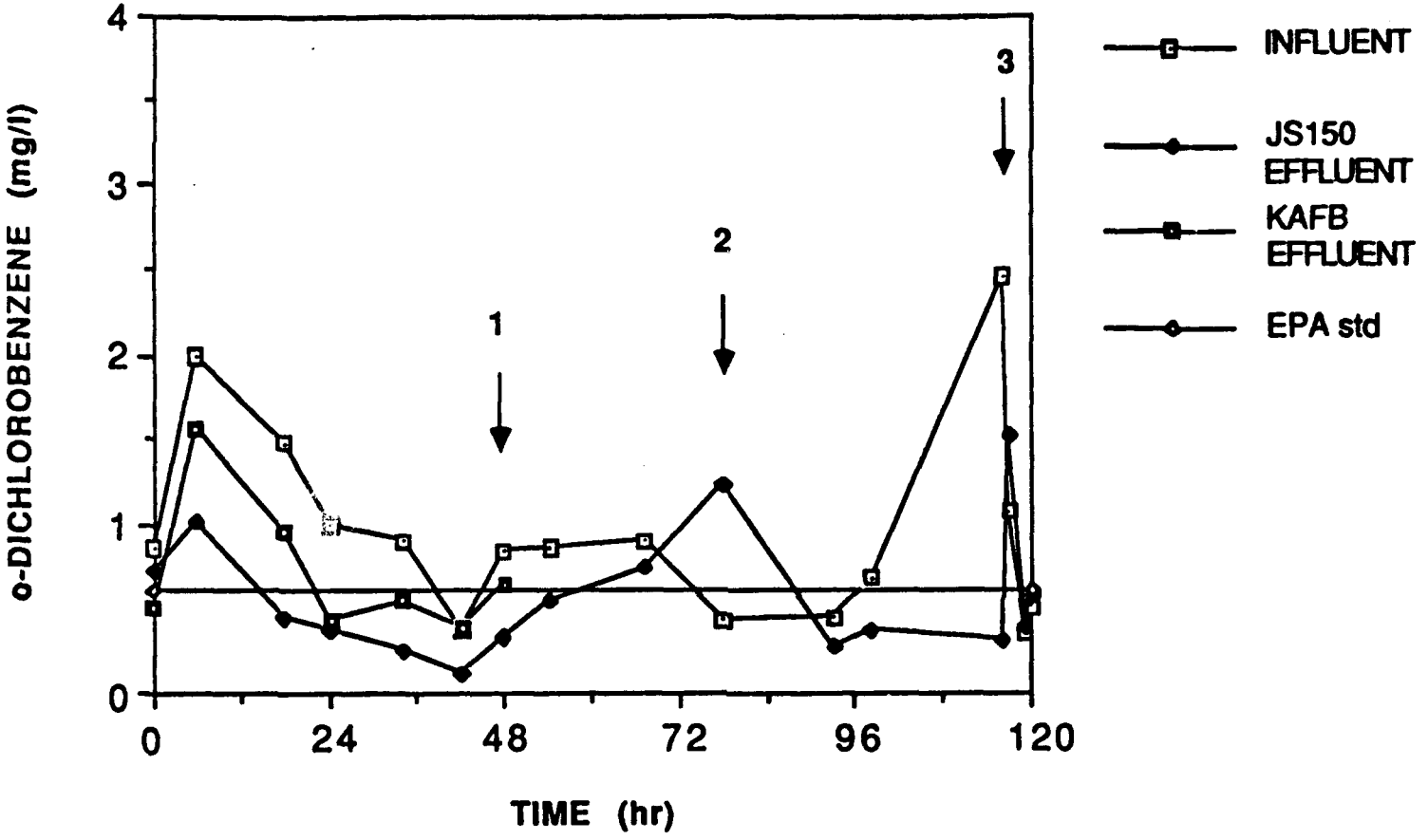

Figure 8. *o*-Dichlorobenzene Removal in the Pilot-Scale Bioreactors. Bioreactors were colonized with either Strain JS150 or the bacteria indigenous to Kelly AFB (KAFB) and operated at 40 minute, 20 minute (Arrow 1), and 30 minute hydraulic residence times (Arrow 2). Dissolved oxygen levels in the feedstock were reduced by turning off air flow to the bioreactor and sparging the holding tank with helium (Arrow 3).

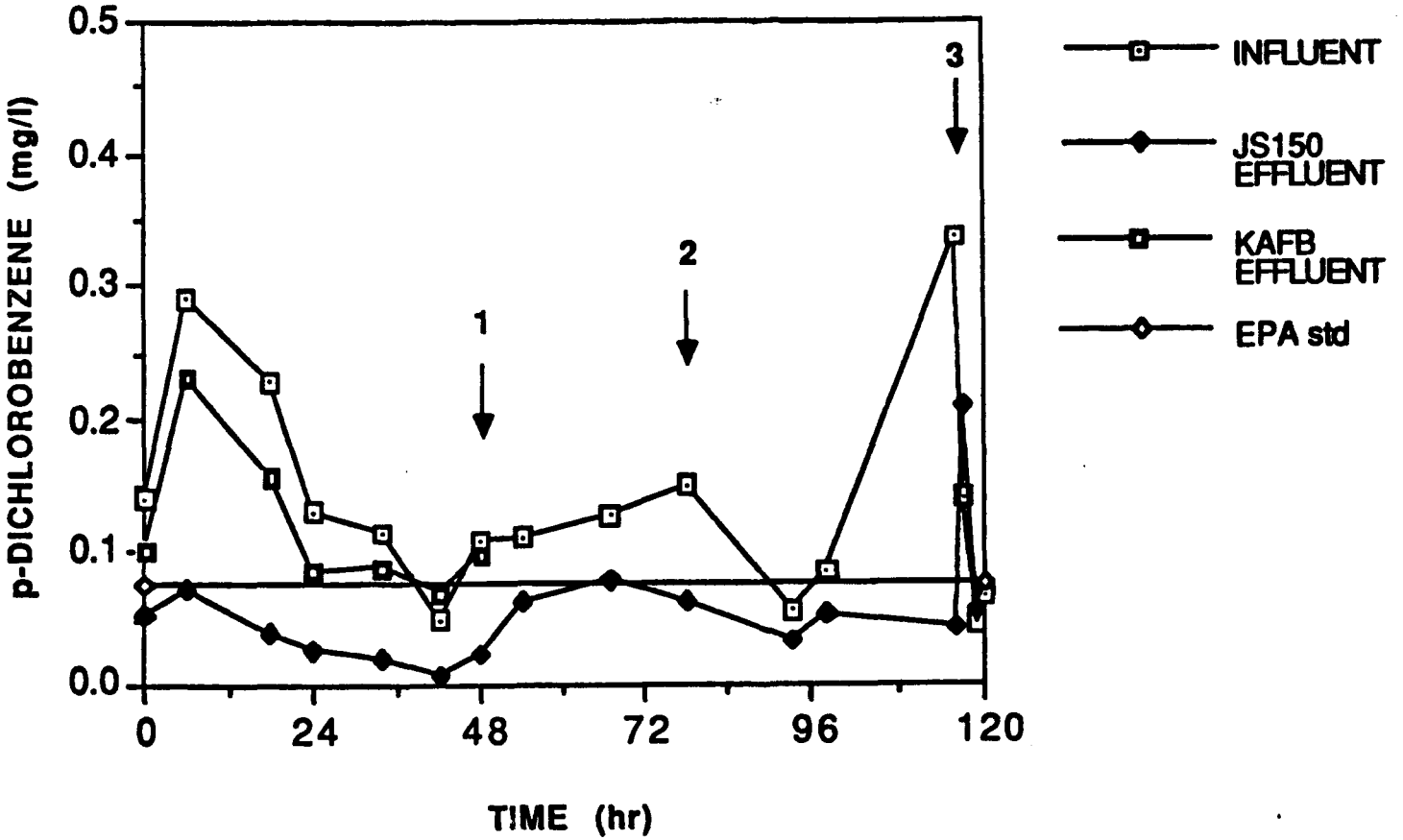

Figure 9. *p*-Dichlorobenzene Removal in the Pilot-Scale Bioreactors. Bioreactors were colonized with either Strain JS150 or the bacteria indigenous to Kelly AFB (KAFB) and operated at 40 minute, 20 minute (Arrow 1), and 30 minute hydraulic residence times (Arrow 2). Dissolved oxygen levels in the feedstock were reduced by turning off air flow to the bioreactor and sparging the holding tank with helium (Arrow 3).

at 40 minutes. In addition, the bioreactor originally colonized with JS150 achieved effluent concentrations below the EPA drinking water limits for each of these compounds (Figures 4-9). Decreasing the residence time to 20 minutes, however, caused the effluent concentrations of the aromatic compounds to exceed the EPA limits. The biodegradative capacity of the bioreactor was restored when the residence time was increased to 30 minutes (Figures 4-9). When the oxygen supply was halted, breakthrough of all the aromatic compounds was immediate (Figures 4-9). These results indicated that biodegradation was the major fate of these compounds.

Microbiological analysis of the pilot-scale bioreactor indicated that Strain JS150 initially represented 42 percent of the initial chlorobenzene degrading population and only decreased to 30 percent following 7 days of continuous operation (Table 6). However, upon careful examination of the phenotypes of bacteria exhibiting chlorobenzene and naphthalene degradation, the typical JS150 profile was not observed (Appendix C). The assumption that the percentage of chlorobenzene degraders that were also naphthalene degraders would provide an estimate of the prevalence of Strain JS150 appears to be in error. This assumption was partly flawed due to the appearance of "false positive" results where some colonies scored positive for growth on MSB plates with chlorobenzene or naphthalene as sole carbon source were not degrading the aromatic compound. In light of these observations the difference in aromatic compound removal efficiencies reported for the two pilot-scale bioreactors may not be related to differences in inocula. Despite the apparent displacement of Strain JS150, however, horizontal gene transfer could explain these results. The JS150 isolate has been noted to harbor several plasmids that could be involved in the degradation of aromatic compounds and may facilitate gene transfer from one organism to another. An example of interspecific gene transfer in a continuous-flow environment was documented by Schmidt (26).

TABLE 6. ENUMERATION OF BACTERIA FROM THE PILOT-SCALE BIOREACTOR. Total counts (nutrient agar) and chlorobenzene degraders were measured in samples from the pilot-scale bioreactors.

	CFU ($x10^6$) per gram		
	Nutrient agar	Chlorobenzene	Naphthalene (%)
Reactor 1 (JS150)			
Batch[a]			
bottom	66.1	52.5	46
top	38.9	30.2	38
Continuous[b]			
bottom	36.3	9.6	32
top	67.6	8.2	28
Reactor 2 (Indigenous)			
Batch[a]			
bottom	4.9	2.0	6
top	2.1	1.2	4
Continuous[b]			
bottom	10.8	9.6	8
top	10.7	8.3	6

[a]Samples taken following batch "fill and draw" phase.

[b]Samples taken following 7 days of continuous operation.

SECTION V

CONCLUSIONS

The objective of this study was to test the ability of submerged fixed-film bioreactors to degrade mixed aromatic contaminants in groundwater. Based on the results of this study, the aerobic fixed-film process is very effective for degrading low concentrations of aromatic contaminants in groundwater. Both the bench- and pilot-scale studies using site S1 groundwater indicated that bioremediation was effective and that the oxygen requirement was critical. These results suggest that natural in situ biodegradation is occurring but is limited by oxygen influx.

In this study we evaluated the efficiency of biodegradation and survival of a pure culture, Strain JS150, as compared with indigenous groundwater bacteria colonizing the bioreactors. This approach was adopted in part due to the potential biochemical road blocks associated with the simultaneous biodegradation of chloro- and methylaromatic compounds (23), and the finding that Strain JS150 can bypass these problems (24). The bioreactor colonized with Strain JS150 was more efficient at removing highly substituted compounds, such as para-dichlorobenzene, but Strain JS150 was displaced by indigenous microorganisms at Site S1 following continuous-flow operation. This result may have been caused by the high concentrations of contaminants, especially ortho-dichlorobenzene which does not support growth of Strain JS150 and is only cometabolized in the presence of other chloroaromatic compounds. Preliminary evidence suggests that ortho-dichlorobenzene is also toxic to JS150. Strain JS150 might have persisted under different operating conditions, such as continuous-flow operation using the dilute chlorobenzene plume associated with Site S1. These results indicate that inoculation with a pure culture was not necessary in this situation. The bacteria indigenous to site S1 groundwater were capable of degrading mixtures of chloro- and methylaromatic compounds. Inoculation might be necessary in situations involving recent release of chlorobenzene or related compounds.

SECTION VI

RECOMMENDATIONS

Based on the results and observations made during the bench- and pilot-scale experiments with groundwater from Site S1, we conclude that all of the contaminants can be biodegraded by indigenous microorganisms. Therefore, we recommend that bioremediation be considered for cleanup of this and other similar sites. In contrast to the statements of Senko et al. (24), our results indicate that the chloroaromatic compounds contaminating Site S1 can be readily degraded, along with the nonhalogenated aromatic compounds. The bioremediation of Site S1 could involve *in situ* and "pump-and-treat" strategies.

We recommend that an *in situ* approach be considered for cleanup of the former depression area. The inability to continuously pump groundwater from this area will be problematical for any "pump-and-treat" approach. Our results indicate that bacteria indigenous to Site S1 can degrade the contaminants at this site if supplied with oxygen. An *in situ* approach that would satisfy this limitation, should facilitate the desired remediation of this site.

Where a "pump-and-treat" technology is possible, such as the chlorobenzene plume at Site S1, we recommend that a fixed-film bioreactor be considered for removal of the contaminants. A logical next step would be to conduct a direct comparison of the fixed-film bioreactor technology with other approaches, such as "air stripping," at a pilot scale. The presence of indigenous bacteria able to degrade chlorobenzene suggests that biodegradation may be removing the contaminant in the area of the chlorobenzene plume at a low rate, without intervention. The degradation rate could be expected to be limited by the influx of oxygen in the recharge water. Continued monitoring might reveal a gradual disappearance of the chlorobenzene plume due to natural biodegradation.

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APPENDIX A

SUMMARY OF

LABORATORY DATA

SUMMARY OF LABORATORY DATA

	TIME(hr)	DATE	pH(in)	pH(out)	DO(in)[a]	DO(out)	FEED(cb)[b]	EFFL(cb)	TEMP(C°)	RES.TIME(min)
1	0.000	2/13/91	6.990	6.850	6.500	5.200	0.000	0.000	22.000	30.000
2	20.000	2/14/91	6.980	6.890	8.800	7.100	2.170	0.029	19.000	30.000
3	44.000	2/15/91	6.980	6.910	9.300	7.900	1.353	0.031	17.000	30.000
4	70.000	2/16/91	6.980	6.940	10.200	8.900	1.218	0.004	12.000	30.000
5	94.000	2/17/91	6.980	6.940	9.100	8.000	1.127	0.014	18.000	30.000
6	114.000	2/18/91	6.980	6.940	8.900	7.900			19.000	30.000
7	120.000	2/19/91	6.970	6.800	6.500	2.000	3.437	0.493	22.000	30.000
8	144.000	2/20/91	6.880	6.820	14.600	2.500	3.618	0.090	22.000	30.000
9	168.000	2/21/91	6.950	6.860	15.800	2.200	3.618	0.382	22.000	30.000
10	196.000	2/22/91	6.960	6.870	12.400	3.400			21.000	30.000
11	212.000	2/22/91	6.950	6.870	14.800	2.100	2.169	0.404	20.000	30.000
12	214.000	2/22/91	6.950	6.870	15.000	2.000			20.000	30.000
13	215.000	2/22/91	6.950	6.870	5.900	1.800	2.118	0.404	20.000	30.000
14	217.000	2/22/91	6.950	6.920	4.400	1.800	1.912	1.360	21.000	30.000
15	219.000	2/22/91	6.960	6.940	3.800	1.800	1.985	1.764	21.000	30.000
16	238.000	2/23/91	6.960	6.870	15.800	1.900	1.733	0.389	19.000	30.000
17	262.000	2/24/91	6.960	6.890	14.900	2.000			20.000	30.000
18	286.000	2/25/91	7.000	6.870	13.000	2.000	1.400	0.223	18.000	50.000
19	310.000	2/26/91	6.970	6.870	13.400	2.100	0.930	0.074	19.000	50.000
20	334.000	2/27/91	6.960	6.880	11.500	2.200	1.130	0.095	19.000	40.000
21	362.000	2/28/91	6.980	6.860	10.900	2.000	1.518	0.140	21.000	40.000

Note: [a]DO = Dissolved Oxygen in mg/L
[b]cb = Chlorobenzene Concentration in mg/L

APPENDIX B

SUMMARY OF FIELD DATA

APPENDIX B

	TIME (hr)	FEED (ben)[b]	FEED (tce)[c]	FEED (tol)[d]	FEED (cb)[e]	FEED (o-dcb)[f]	FEED (p-dcb)[g]
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	2.500	0.498	0.406	0.348	2.458	3.872	0.463
3	22.000	0.108	0.135	0.079	0.403	0.446	0.055
4	29.000	0.138	0.193	0.116	0.473	0.521	0.065
5	56.000	0.011	0.016	0.030	0.103	0.188	0.024
6	71.000	0.204	0.358	0.199	0.919	1.318	0.163
7	80.000	0.069	0.079	0.092	0.743	1.176	0.147
8	93.000	0.148	0.166	0.153	0.877	0.823	0.111
9	98.000	0.093	0.149	0.097	0.409	0.499	0.072
10	102.000	0.240	0.355	0.253	1.199	1.293	0.180
11	115.000	0.049	0.076	0.104	0.373	0.359	0.037
12	124.000	0.124	0.190	0.153	0.865	0.868	0.140
13	130.000	0.063	0.056	0.239	1.235	1.998	0.282
14	142.000	0.037	0.024	0.161	1.220	1.479	0.228
15	148.000	0.035	0.039	0.094	0.852	1.000	0.131
16	158.000	0.064	0.069	0.286	0.832	0.891	0.115
17	166.000	0.057	0.085	0.108	0.551	0.383	0.048
18	172.000	0.145	0.238	0.172	0.842	0.834	0.108
19	178.000	0.151	0.248	0.192	0.957	0.867	0.112
20	191.000	0.164	0.247	0.191	1.107	0.902	0.128
21	202.000	0.017	0.137	0.144	0.813	0.438	0.151
22	217.000	0.009	0.010	0.023	0.535	0.447	0.056
23	222.000	0.018	0.017	0.046	0.650	0.680	0.086
24	240.000	0.346	0.530	0.281	1.435	2.467	0.337
25	241.000	0.152	0.227	0.137	0.735	1.073	0.140
26	243.000	0.042	0.067	0.058	0.481	0.354	0.045
27	244.000	0.066	0.106	0.096	0.599	0.515	0.068

Note: [a] FEED = influent stream

[b] ben = benzene, mg/L

[c] tce = trichloroethylene, mg/L

[d] tol = toluene, mg/L

[e] cb = chlorobenzene, mg/L

[f] o-dcb = o-dichlorobenzene, mg/L

[g] p-dcb = p-dichlorobenzene, mg/L

[h] JS150 = effluent from JS150 reactor

[i] KAFB = effluent from indigeneous microbe reactor

[j] DO = dissolved oxygen, mg/L

	JS150 (ben)[h]	JS150 (tce)	JS150 (tol)	JS150 (cb)	JS150 (o-dcb)	JS150 (p-dcb)	KAFB[1] (ben)
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.013	0.029	0.052	0.075	0.375	0.014	0.004
3	0.003	0.033	0.023	0.023	0.117	0.003	0.001
4	0.032	0.158	0.023	0.080	0.340	0.015	0.007
5	0.001	0.005	0.019	0.029	0.162	0.004	0.002
6	0.121	0.298	0.127	0.541	0.960	0.097	0.055
7	0.012	0.011	0.046	0.177	1.306	0.085	0.002
8	0.007	0.079	0.019	0.094	0.623	0.061	0.003
9	0.003	0.033	0.014	0.034	0.431	0.046	0.001
10	0.013	0.162	0.064	0.202	0.683	0.060	0.030
11	0.003	0.029	0.050	0.070	0.359	0.020	0.001
12	0.002	0.063	0.025	0.051	0.726	0.053	0.001
13	0.004	0.016	0.066	0.129	1.019	0.073	0.003
14	0.002	0.005	0.028	0.054	0.441	0.039	0.002
15	0.004	0.004	0.055	0.077	0.368	0.025	0.004
16	0.002	0.002	0.043	0.055	0.252	0.020	0.002
17	0.003	0.005	0.036	0.071	0.121	0.006	0.002
18	0.002	0.021	0.014	0.040	0.339	0.023	0.003
19	0.008	0.112	0.060	0.223	0.551	0.061	
20	0.006	0.119	0.055	0.220	0.738	0.079	
21	0.067	0.101	0.138	0.356	1.223	0.062	
22	0.001	0.002	0.011	0.019	0.281	0.034	
23	0.005	0.002	0.052	0.079	0.361	0.052	
24	0.008	0.010	0.049	0.083	0.309	0.043	
25	0.137	0.369	0.139	0.857	1.525	0.209	
26	0.054	0.080	0.082	0.523	0.386	0.056	
27	0.074	0.116	0.073	0.646	0.570	0.075	

	KAFB (tce)	KAFB (tol)	KAFB (cb)	KAFB (o-dcb)	KAFB (p-dcb)	FEED DO	JS150 DO
1	0.000	0.000	0.000	0.000	0.000		
2	0.019	0.005	0.025	0.327	0.005	5.600	1.400
3	0.025	0.024	0.017	0.128	0.005	9.400	1.500
4	0.094	0.029	0.053	0.356	0.023	8.200	0.700
5	0.002	0.028	0.052	0.193	0.003	9.500	1.700
6	0.225	0.097	0.485	0.906	0.103	6.500	0.500
7	0.020	0.033	0.059	0.898	0.105	11.000	3.800
8	0.032	0.019	0.059	0.516	0.084	10.300	1.400
9	0.030	0.014	0.014	0.168	0.035	9.500	0.800
10	0.223	0.074	0.469	1.160	0.138	5.500	5.100
11	0.002	0.049	0.047	0.066	0.018	6.100	4.400
12	0.017	0.025	0.037	0.511	0.100	5.500	4.500
13	0.013	0.055	0.123	1.568	0.232	8.400	10.200
14	0.004	0.032	0.073	0.963	0.156	7.700	9.500
15	0.003	0.045	0.079	0.423	0.086	6.600	7.700
16	0.007	0.027	0.059	0.550	0.087	7.100	8.000
17	0.015	0.025	0.057	0.392	0.070	8.200	8.700
18	0.044	0.031	0.105	0.640	0.097	6.200	7.700
19						6.700	6.400
20						6.700	5.500
21						6.500	5.100
22						7.400	6.400
23						6.200	6.800
24						5.800	6.000
25						5.000	1.500
26						5.200	0.700
27						5.000	0.700

	KAFB DO	FEED pH	JS150 pH	KAFB pH	TEMPERATURE (°C)
1					
2	1.300				
3	3.500				
4	0.800	6.890	6.850	6.850	22.800
5	4.900	7.090	6.890	6.990	24.300
6	0.500				24.800
7	6.600	7.240	7.150	7.220	23.500
8	4.400	6.940	6.800	6.870	22.700
9	1.500	7.330	7.200	7.240	25.200
10	3.600	7.240	7.180	7.160	24.000
11	4.200	7.280	7.010	7.080	23.600
12	3.300				
13	9.800	7.180	6.910	6.890	20.100
14	8.000	6.980	6.860	6.930	20.000
15	6.000	7.260	7.140	7.200	23.500
16	6.500	6.850	6.820	6.810	20.900
17	7.900	7.050	6.850	6.910	19.500
18	5.630	7.110	7.040	7.040	22.500
19		7.240	7.190		20.200
20		7.140	7.070		19.900
21		7.210	7.060		20.000
22		7.120	6.950		20.800
23		7.100	7.040		21.000
24		6.980	6.960		20.200
25		7.010	6.890		21.000
26		7.180	7.050		20.900
27		7.120	7.040		20.900

APPENDIX C

GROWTH ON

SELECTED SUBSTRATES

GROWTH ON SELECTED SUBSTRATES

Strain[a]	Colony[b]	BEN	TOL	CB	pDCB	oDCB	mDCB	NAP	pCR
1600	A	-	-	-	-	-	+	-	-
1602B	C	-	+	+	-	-	-	-	-
1603	E	+	-	+	+	-	-	-	-
1604	F	+	-	+	+	-	-	-	-
1606	D	-	+	+	-	-	-	-	-
1608B	E	-	-	-	-	+	-	-	-
1613	D	-	+	-	-	-	-	-	-
1614	G	+	-	+	+	-	-	-	-
1619A	E	-	-	+	-	-	-	-	-
1627A	E	+	-	+	-	-	-	-	-
1628	F	+	-	-	-	-	-	-	-
1633B	H	-	+	+	-	-	-	-	-
1(5	F	+	-	+	-	-	-	-	-
1636	I	-	-	-	-	-	-	+	+
1637B	E	-	-	-	-	+	-	+	+
1641A	E	-	-	-	-	-	-	-	+
1642B	I	-	-	-	+	-	-	+	-
JS150	J	-	+	+	-	-	-	+	-

[a]Selected strains were initially grown in the presence of chlorobenzene, then isolated and tested for growth on minimal agar plates with substrates provided in the vapor phase.

[b]Colony type: A = yellow, smooth; C = tan, pebbled; D = tan, dry; E = beige, wrinkled; F = beige, radial zones; G = yellow, radial zones; I = pink, rough; J = tan, smooth.

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